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A new species of *Podosphaera* sect. *Sphaerotheca* from China

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Abstract — *Podosphaera girardiniae* (*Erysiphaceae*, *Podosphaera* sect. *Sphaerotheca*), identified in China on *Girardinia suborbiculata*, is described, illustrated, and compared with allied species. The phylogeny of this new species has been inferred from internal transcribed spacer (ITS) and 28S rDNA sequence analyses.

Key words — molecular phylogeny, morphology, taxonomy

Introduction

In the autumn of 2013, *Girardinia suborbiculata* (*Urticaceae*) was identified in Shaanxi, China, that was heavily infected by a powdery mildew. Based on the asexual morph characterized by catenescent conidia with distinct fibrosin bodies and chasmothecia with a single ascus and mycelioid appendages, this collection was easily assignable to *Podosphaera* sect. *Sphaerotheca* (Lév.) de Bary, as currently circumscribed and recognized (Braun & Cook 2012). This section consists of 75 species, with 57% of the host plants belonging to the *Rosaceae* (Takamatsu et al. 2010). Given the relatively large peridial cells, the powdery mildew on *Girardinia* can be placed in *Podosphaera* subsect. *Magnicellulatae* (U. Braun) U. Braun & Shishkoff (Braun & Cook 2012).

Podosphaera parietariae (Schwarzman) U. Braun & S. Takam. on *Parietaria* spp. in Asia and Europe is the only species on an urticaceous host assigned to *Podosphaera* sect. *Sphaerotheca,* where it has been placed based on its small (8–25 µm diam.) peridial cells (Braun 1987, Braun & Takamatsu 2000, Braun & Cook 2012). Its morphology easily eliminates *P. parietariae* as the causal agent of the *Girardinia* infection in China. However, in subsect. *Magnicellulatae,* plurivorous races must be taken into consideration, particularly the *P. xanthii* (Castagne) U. Braun & Shishkoff complex to which the collection on *Girardinia*

appears to belong. Therefore, it was necessary to perform molecular sequence analyses to elucidate the taxonomic status of the powdery mildew involved.

Materials & methods

Living leaves of *Girardinia suborbiculata* bearing the holomorph of a powdery mildew were collected in October 2013 in the Qinling Mountains within Lueyang County in China. Herbarium specimens were deposited in the Mycological Herbarium of Forestry College, Northwest A & F University, Yangling, Shaanxi Province, China (HMNWAFU-CF) and the herbarium of Martin Luther University, Halle (Saale), Germany (HAL).

The specimen was mounted in distilled water and examined using light microscopy (Olympus, CX31RTSF, Japan). The teleomorphic features of the fungus, including chasmothecia, appendages, asci, and ascospores, were described, measured, and photographed. A scanning electron microscope (JEOL, JSM-6360LV) was used to observe the anamorph ultrastructure, particularly the surface features of conidia (Cook et al. 1997) and appressoria of this fungus; SEM images were taken.

Genomic DNA was extracted from chasmothecia using Chelex-100 (Walsh et al. 1991; Hirata & Takamatsu 1996). The ITS region of the nuclear rDNA (including 5.8S and 28S rDNA sequences with domains D1 and D2) were amplified via polymerase chain reaction (PCR) using primers designed for each region: ITS1 and ITS4 (White et al. 1990) were used to amplify the ITS region, while LSU1 and LSU2 (Scholin et al. 1999) were used to amplify the 28S rDNA sequence.

The PCR assays were conducted in a 50 μL final volume (Hirata & Takamatsu 1996) containing 27 μL of 2× BoisTaq PCR MasterMix, 1 μL of each of primer, 1 μL of the extracted DNA and 20 μL of ddH2O (Hirata & Takamatsu 1996). Thermal cycling in a PTC-200 thermal cycler (BioRad) comprised an initial denaturation step at 95°C for 5 min, 35 cycles of 94°C for 1 min + 60°C for 1 min + 72°C for 1 min, and a final elongation step at 72°C for 8 min. A negative control for each set of reactions replaced template DNA with ddH_2O. The PCR products were separated by electrophoresis on a 2% agarose gel in TAE buffer and purified using the Zymoclean™ Gel DNA Recovery Kit, according to the manufacturer's instructions. The purified DNA products were ligated into the pMD18-T vector (Takara) and transformed into *E. coli* DH5α cells. The cloned fragments were sequenced by Sangon Biotech (Shanghai) Co., Ltd.

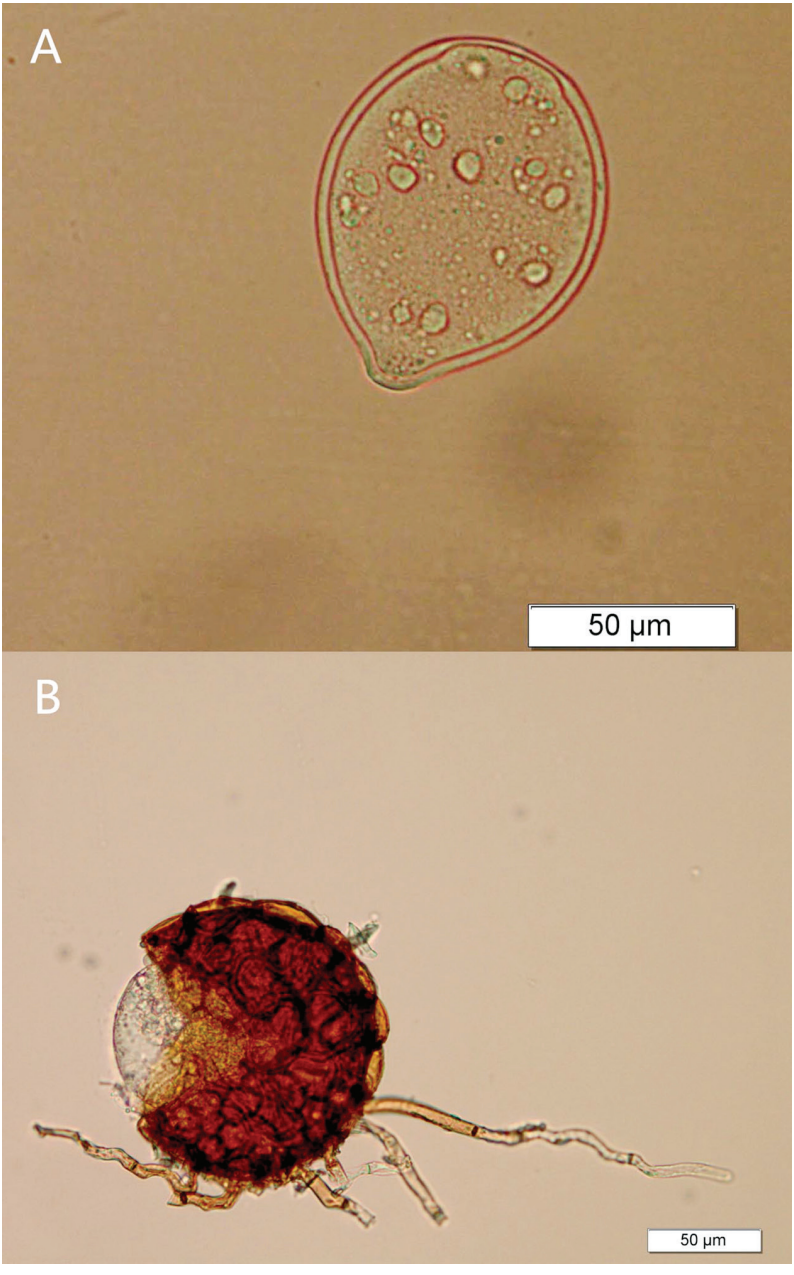
All DNA sequences were aligned using Clustal X 1.81 (Thompson et al. 1997), and the alignments were adjusted following Nei & Kumar (2000). All positions containing gaps or missing data were eliminated from the dataset. Cladistic trees were constructed using the neighbor-joining method with the Kimura 2-parameter substitution model in MEGA 4.0 (Tamura et al. 2007). Branch robustness was assessed by bootstrap analysis with 1,000 replicates.

Taxonomy

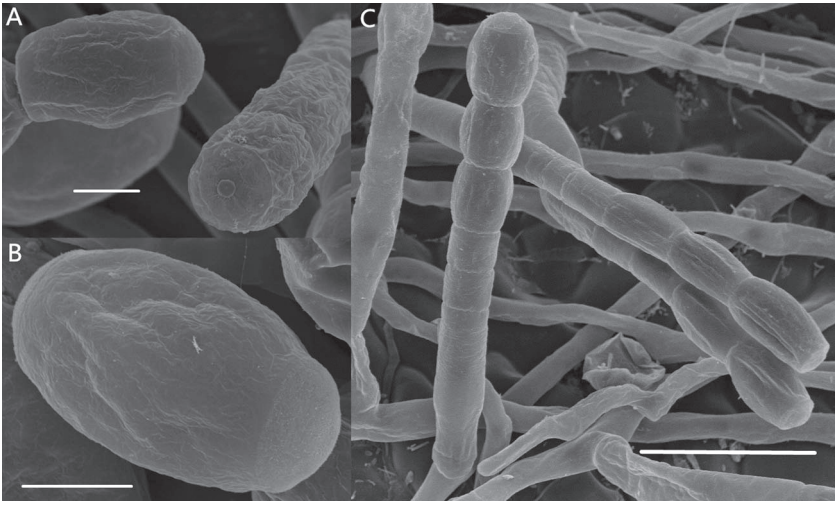
Podosphaera girardiniae Z.M. Cao & L.C. Bai, **sp. nov.** Plates 1–3

MycoBank MB 810810

Morphologically and phylogenetically similar to *Podosphaera xanthii* but sufficiently distinct genetically to be considered a separate species.



Pl. 1. *Podosphaera girardiniae* (Holotype). A. asci; B. chasmothecia and appendages.

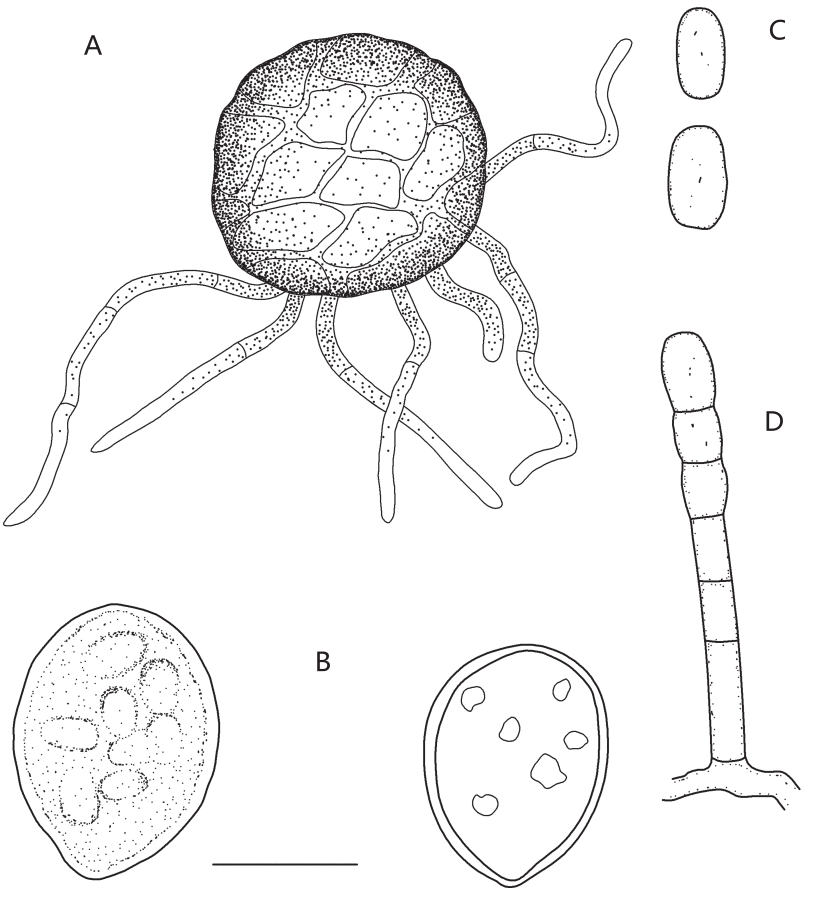


Pl. 2. *Podosphaera girardiniae* (Holotype). A. conidia and conidiophore; B. conidium; C. conidiophore, conidia, and hyphae. Scale bars: A, B = 10 μm; C = 50 μm.

Type: China, Shaanxi, Qinling Mountains, Lueyang County, 33°19′29″N 106°08′29″E; alt. 840 m, on living leaves of *Girardinia suborbiculata* C.J. Chen (*Urticaceae*), Oct. 2013, L.C. Bai (**Holotype**, HMNWAFU-CF 2013125; **isotype**, HAL 2650 F; GenBank KJ540945, KJ540944).

Etymology: referring to the host genus.

Mycelium mainly foliicolous, amphigenous, effuse or in irregular patches, later confluent, often covering the entire leaf surface, white, persistent. Hyphal appressoria indistinct to slightly nipple-shaped. Hyphae smooth or almost smooth, 3–8 μm wide, colorless. Conidiophores arising from the upper surface of hyphal mother cells, erect, straight to somewhat curved, foot-cells cylindrical, about 34–76(–92) × 9–15 μm, followed by 1–3 shorter cells, forming catenescent conidia. Conidia ellipsoid-ovoid, doliiform, 25–36 × 15–21 μm, with a length/width ratio of 1.4–2.1. Chasmothecia mainly epiphyllous, gregarious, 82–133 μm diam. , subglobose; peridium cells conspicuous, large, irregularly shaped, 14–44 μm diam, appendages usually few, about 2–14, in the lower half, mycelioid, simple, unbranched, sometimes interlaced with each other, about 0.4–3.9 times as long as the chasmothecial diam. (up to about 410μm), 5–9 μm wide, brown below and paler towards the tip or most of the short appendages brown throughout, 0–4-septate, walls thin, smooth or almost so; ascus broadly ovoid or broadly ellipsoid-subglobose, 71–96 × 61–77 μm, almost sessile, terminal oculus 15–22 μm diam., wall 1.5–3 μm thick,



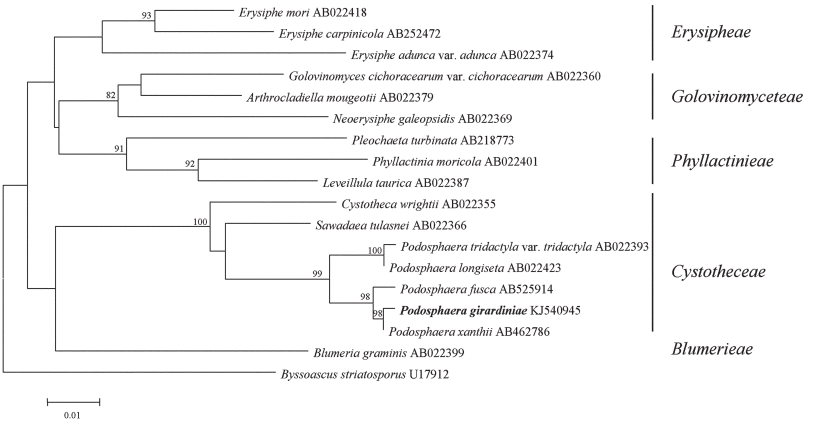
Pl. 3. *Podosphaera girardiniae* (Holotype). A. chasmothecia and appendages; B. asci; C. conidia; D. conidia and conidiophore. Scale bars = 50 μm.

6–8-spored; ascospores broadly ellipsoid-ovoid, 16–23 × 13–17 μm, colorless, development relatively late.

Phylogeny

28S analysis Pl. 4

The 28S rDNA sequence comprised 632 total characters and was deposited in GenBank under accession number KJ540945. The sequence was aligned



Pl. 4. Neighbor-joining tree based on distances derived from sequences of the 28S rRNA genes from 17 taxa of *Erysiphaceae,* with *Byssoascus striatosporus* as outgroup. The bar indicates a distance of 0.01.

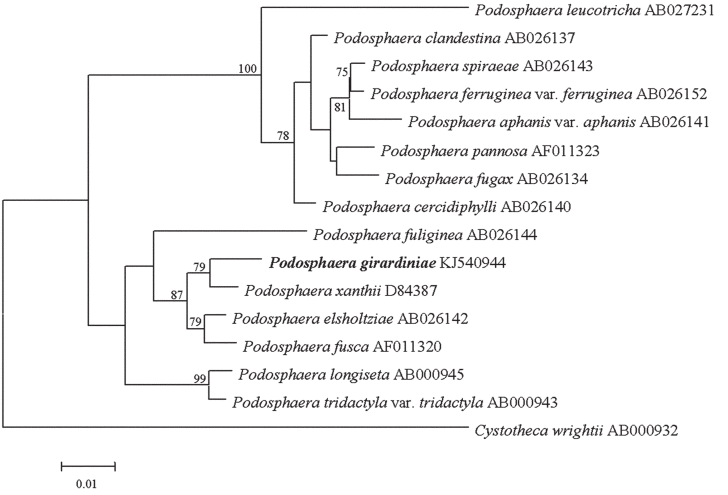
with 15 sequences representing the five tribes of *Erysiphaceae*. The 28S rDNA phylogenetic tree places the *Podosphaera girardiniae* sequence in a strongly supported clade (bootstrap value = 98%) where it clusters with *P. xanthii*, clearly supporting its placement in the genus *Podosphaera*, probably within subsect. *Magnicellulatae*. *Byssoascus striatosporus* (G.L. Barron & C. Booth) Arx was used as outgroup.

ITS analysis Pl. 5

The ITS rDNA sequence analysis comprised 499 total characters and was deposited in GenBank under accession number KJ540944. The sequences were aligned with 14 sequences representing *Podosphaera* Kunze emend. U. Braun & S. Takam. *Cystotheca wrightii* Berk. & M.A. Curtis was used as outgroup. The ITS phylogenetic tree placed *Podosphaera xanthii* and *P. girardiniae* in one clade with 79% bootstrap support. The *P. girardiniae* sequences differ from all available sequences representing other *P.* sect. *Sphaerotheca* species, including *P. xanthii*. The phylogenetic analyses of the rDNA sequences indicated that *Podosphaera* on *Girardinia suborbiculata* represents a new species.

Discussion

The only other *Podosphaera* known on an urticaceous host, *P. parietariae*, clearly differs morphologically from *P. girardiniae,* and its chasmothecia



Pl. 5. Neighbor-joining tree based on distances derived from the ITS1, ITS2, and 5.8S rRNA gene sequences from 15 *Podosphaera* taxa, with *Cystotheca wrightii* as outgroup. The bar indicates a distance of 0.01.

with small peridial cells place it in subsect. *Sphaerotheca*. Our phylogenetic analyses support a close affinity of *P. girardiniae* with *P. xanthii*, from which it is morphologically barely distinguishable. Nonetheless the genetic differences are too significant to assign the *Girardinia* powdery mildew to *P. xanthii*. The sequence selected for *P. xanthii* was previously used by other authors (e.g., Kousik et al. (2011), who demonstrated that this sequence and other sequences of *P. xanthii* from several cucurbits, beans and exotic impatiens species share 100% ITS similarity). These genetic differences support the *Girardinia* powdery mildew as a new species.

Acknowledgments

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